Journal of Forestry Research (2008) 19(1):37–43
DOI: 10.1007/s11676-008-0006-7

RESEARCH PAPER

Temperature dependence of nitrogen mineralization and microbial status in O_H horizon of a temperate forest ecosystem

Ali Bagherzadeh [1]*, Rainer Brumme [2], Friedrich Beese [2]

[1]*Department of Agriculture*, *Azad University of Mashhad*, *Emamyeh Boulevard*, *P.O Box:* 91735-413, *Mashhad, Iran*
[2]*Institute of Soil Science and Forest Nutrition, Georg-August-University of Goettingen*, *Buesgenweg* 2, 37077 *Goettingen*, *Germany*

Abstract: It was hypothesized that increasing air and/or soil temperature would increase rates of microbial processes including litter decomposition and net N mineralization, resulting in greater sequestration of carbon and nitrogen in humus, and consequently development in O_H horizon (humus horizon). To quantify the effect of temperature on biochemical processes controlling the rate of O_H layer development three adjacent forest floors under beech, Norway spruce and mixed species stands were investigated at Solling forest, Germany by an incubation experiment of O_H layer for three months. Comparing the fitted curves for temperature sensitivity of O_H layers in relation to net N mineralization revealed positive correlation across all sites. For the whole data set of all stands, a Q_{10} (temperature sensitivity index) value of 2.35–2.44 dependent on the measured units was found to be adequate for describing the temperature dependency of net N mineralization at experimental site. Species-specific differences of substrate quality did not result in changes in biochemical properties of O_H horizon of the forest floors. Temperature elevation increased net N mineralization without significant changes in microbial status in the range of 1 to 15ºC. A low C_{mic} /C_{org} (microbial carbon/organic carbon) ratio at 20°C indicated that the resource availability for decomposers has been restricted as reflected in significant decrease of microbial biomass.

Keywords: beech; spruce; nitrogen mineralization; forest floor; temperature; temperature sensitivity index (Q_{10})

Introduction

Temperature is a key factor that regulates many terrestrial biochemical processes, such as soil respiration (Raich et al. 1992), litter decomposition (Jansson et al. 1985; Hobbie 1996), net N mineralization and nitrification (MacDonald et al. 1995). Organic matter is of primary importance for nutrient availability and the sustainability of forest productivity. Essential nutrients are stored in organic matter like nitrogen and base cations and are available for plant uptake after mineralization. However, organic matter accumulation in forest floor can affect the forest ecosystem by immobilizing nutrients making them unavailable for plant uptake. The humus forms are useful indicators for estimating the immobilization and availability of nutrients especially nitrogen in forest soils. The immobilization increase from mull humus to moder, or mor is a consequence of local ecological conditions particularly the climate, vegetation type. Mull humus indicates a better nutrient availability, stores only 0.3 $Mg \cdot ha^{-1}$ (simplified expression of Mg (nitrogen)·ha^{-1}) in contrast to mor humus with 1.3 $Mg \cdot ha^{-1}$ estimated on 600 forest soil profiles in Germany (Wolff et al. 1997). The present increase of organic matter in the forest floor from mull to mor humus indicates an exponentially increase in the O_H horizon while the $O_L/_F$ (litter/fermentation) horizons follows an hyperbolic curve (BZE-data-set, Wolff et al. 1997). The O_L/O_F horizons were found to be the horizons with highest microbial activity and the hyperbolic increase suggests that with increasing soil acidity from mull to mor humus, the microorganisms build up a new decomposer refuge on the top of the mineral soil which is in the steady state between litter input and decomposition. In contrast to the O_L/O_F horizons the O_H horizon shows a potentially unlimited accumulation from mull to mor humus. Formation of recalcitrant humic compounds by humification in the O_F horizon and their sequestration in the O_H horizon seems to be the prevailing process in thickening this horizon. The unlimited C accumulation in this horizon indicated that microbial decomposition did not equal the production of recalcitrant material. To better understand why the O_H horizon shows an exponential increase it is important to know the processes involved in mineralization of nitrogen in this horizon and the factors that control the rate of this processes. Nitrogen transformations involve biological processes that are temperature dependent. In particular temperature controls on net N mineralization has attracted special atten-

Foundation project: The research was financially supported by Institut für Bodenkunde und Waldernährung, Georg-August-Universität, Göttingen, Germany.

Received: 2007-09-11; Accepted: 2007-11-05

The online version is available at http://www.springerlink.com

Biography: Ali Bagherzadeh (1966-), *Corresponding author, male, Associate Professor in Department of Agriculture, Azad University of Mashhad, Emamyeh Boulevard, P.O Box: 91735-413, Mashhad, Iran. (E-mail: abagher_ch@yahoo.com)

Responsible editor: Zhu Hong

tion because of the importance of available N for meeting microbial demands for an element that is usually considered as limiting in soils (Aber et al. 1991). The temperature response of N mineralization has been shown to follow the Q_{10} function (Kladivko et al. 1987; Dalias et al. 2002) with a Q_{10} of net N mineralization close to 2. Variations of temperature responses are not, however, extensively investigated and controls of substrate quality (Campbell et al. 1984; De Neve et al. 1996) are not explicitly understood. Microbial biomass and activities are important ecosystem characteristics to be used for predicting rates of nitrogen mineralization. Both microbial biomass and activities can be affected by changing soil temperature regimes (Nadelhoffer et al. 1991; Ellert et al. 1992). Reports in the literature indicate that soil microbial biomass and activity may increase in response to temperature elevation (Sprent 1987; Lioyd et al. 1994) and can lead to an increase in net N mineralization and therefore an increase in nutrient availability to plants.

The present study aimed to characterize the effects of temperature as a major controlling factor on microbial biomass status and net N mineralization in O_H horizon of the three adjacent stands at Solling forest, Germany. It is of great interest to find the role of temperature elevation on biochemical processes, microbial biomass and net N mineralization, controlling the rate of O_H layer development with respect to different substrate quality of the forest floors.

Materials and methods

Study site

The study area is located at the Solling forest in Lower Saxony, Germany (51°47′N and 9°37′E) at 500 m above see level. The climate is characterized by a mean annual temperature of 6.5°C, a mean annual precipitation of 1050 mm. The annual temperatures range from an average of 14°C in July to an average of -2°C in January. The dominant soil type are slightly podsolic Dystric Cambisol (FAO 1988) developed on Triassic sandstone covered by a layer of loess with a thickness varying from 0.2 m to 2 m (average 80 cm) (Tiktak et al. 1995). Soil texture is dominated by silty loam and the prevailing humus form is typical moder. We conducted our study in November 2006 from three adjacent treatment plots of 20 m × 20 m each with 100 to 120 years old trees, one plot included Norway spruce (*Picea abies* L. karst), another was covered by beech trees (*Fagus sylvatica* L.), and the third plot consisted of both beech and spruce trees. In 1989 a surface application of 2.3-t·ha^{-1} finely ground dolomitic limestone on the forest floor of spruce stand was registered to alter the pH in the organic horizon.

Soil sampling and experimental design

Thirty undisturbed field-moist humus samples with 8.4-cm diameter and 14-cm height were collected randomly at each plot by using a stainless steal auger. Twelve extra soil cores were collected from each stand and by removing the forest floor $O_L/_F$ layers were analyzed for initial measurements of pH, carbon, nitrogen, C/N ratio, microbial biomass and ergosterol content. Samples were placed into incubation vessels (8.4-cm diameter, 14-cm height) and incubated at 1°C, 5°C, 10°C, 15°C, and 20°C with six replications from each stand at each temperature in temperature-controlled incubation chambers for three months. The moisture content of the samples was controlled by continuous weighing over the incubation period.

Soil analyses and statistical calculations

Moisture content was determined in sub-samples after drying at 105°C. Microbial biomass carbon and nitrogen were determined using the chloroform fumigation-extraction method (Brookes et al. 1985, Vance et al. 1987). Twelve fresh sub-samples were taken from each stand. Some six sub-samples were immediately extracted with 0.5-mol/L K_2SO_4 (5:1 ratio of solution to soil dry mass), and the others were fumigated for 3d and then extracted; organic C and N were determined from the extracts.

The Organic C in the K_2SO_4 extracts was analyzed by dry combustion at 680°C using TOC 5050 Shimadzu carbon analyzer (Shimadzu GmbH, Duisburg, Germany). The extracted ammonium, nitrate and total N were analyzed by a continuous flow system spectrophotometer (Skalar Analytic GmbH, D-41812 Erkelenz, Germany). The differences in organic carbon and nitrogen extracted between the fumigated and non-fumigated soils (C and N flushes) are assumed to represent the C and N released from lysed soil microbes. biomass C = E_C/k_{EC}, where E_C is organic C extracted from fumigated soil minus organic C extracted from non-fumigated soil, and k_{EC} is the coefficient of E_C (k_{EC} = 0.45), (Joergensen 1996). Biomass N = E_N/k_{EN}, where E_N is total N extracted from fumigated soil minus total N extracted from non-fumigated soil, and k_{EN} is the coefficient of E_N (k_{EN} = 0.54) (Brookes et al. 1985).

The measurement procedure of microbial biomass C and N in incubated samples after the incubation period was as mentioned above. An initial sub-sample was analyzed to determine total C and N by dry combustion in a CN-auto analyser (Vario, Elementar Analysensysteme, Hanau, Germany) and soil pH by a digital pH-meter (WTW GmbH Wesl-Germany). The quantification of fungal biomass was made by determination of ergosterol content. Ergosterol was determined according to Djajakirana et al. (1996). Moist soil of 1-g dry weight was extracted with 100-ml ethanol for 30 min by oscillating shaking at 250 rev per minute. Ergosterol content was measured by reversed-phase HPLC analysis at 25°C using a column of 12.5-cm Spherisorb ODS II S5 with a mobile phase of 97% vol. methanol/3% vol. water and detection at 282 nm.

The effect of temperature on net nitrogen mineralization was investigated by estimating the rate of increase of extractable ammonium and nitrate in samples after 12 weeks of incubation. Extractable ammonium and nitrate were determined by the extraction of 40-g soil with 200 mL of 0.5-mol·L^{-1} K_2SO_4 (~ 5:1 ratio of solution to dry mass soil). Extraction was done by shaking the samples for one hour and filtering the extracts through K_2SO_4-rinsed filter papers. Extracts were analyzed by a continuous flow system spectrophotometer (Skalar Analytic GmbH,

D-41812 Erkelenz, Germany). An exponential function equation was used to calculate the temperature effects on net nitrogen mineralization by a nonlinear curve fitting procedure which assumes an exponential relationship between the rate of the process under consideration and the temperature, where the b_0 and b_1 as fitting parameters were calculated (Davidson et al. 1998).

$$N_{NM} = b_0 e^{(b1.T)} \quad (1)$$

where, N_{NM} is the net nitrogen mineralization (per measured units), b_0 and b_1 are two fitting parameters and T is the temperature (°C).

The exponential function Q_{10} was used to show temperature sensitivities of a complex of biochemical processes in soil, calculated as:

$$Q_{10} = e^{(10\,b1)} \quad (2)$$

Statistics

Analysis of variance (ANOVA) was tested by Mann-Whitney U-Test at $p<0.05$ level, performed by the program Statistica version 6.0.

Results and discussion

Biochemical characteristics of O_H horizons

The thickness and the mass of forest floor O_H horizon increased slightly from beech over mixed species, to spruce forests but not significantly different between the stands ($p<0.05$). Site variation effects on biochemical characteristics of the humus layers were also negligible as shown in Table 1. The pH $_{(KCl)}$ of the O_H horizons was very low, ranged from 2.96 to 3.21 and represented the overall median of forest floor O_H horizons in Germany of about 3.0 (pH $_{(KCl)}$), (Wolff et al. 1997). The average C/N ratio of 20.8 was lower than the overall median of 24 in German moder humus (Wolff et al. 1997) which indicates a higher N immobilization from nitrogen deposition at Solling forest. Lower soil pH and C/N ratios as a result of high N and acid deposition during the last decades (Ulrich 1994) were indicated by former soils analyses of pH (Hallbäcken and Tamm, 1986; Heisner et al. 2003) and total nitrogen at Solling forest (Zezschwitz 1980; Buberl et al. 1994). The values of C/P mineralization were greater than C/N mineralization ratios, typically by an order of magnitude. This is consistent with other studies showing that microbes can immobilize almost all available phosphate in organic matter (Walbridge et al. 1987). The O_H substrate quality was also found to be similar regarding the content of microbial biomass estimated in a survey of O_H horizons in Lower Saxony, Germany (Anderson 2003). The microbial biomass of the forest soils in Lower Saxony ranged from 1.3 to 2.8 $mg \cdot g^{-1}$ with a high spatial variation of up to 0.9 $mg \cdot g^{-1}$ which was not significantly different to the mean of 3.8 $mg \cdot g^{-1}$ at the present study. As a result of insignificant differences in chemical characteristics of the O_H horizon between beech and Norway spruce in pure and mixed species stands even for the Ca content it can be assumed that the application of lime in 1989 has not significantly changed the O_H horizon at the spruce stand.

Table 1. Mean initial O_H horizon characteristics of the study area at Solling forest, Germany

Stand	Height (cm)	Mass ($Mg \cdot ha^{-2}$)	Moisture (%)	pH (KCl)	pH (H_2O)	C_{org} ($g \cdot kg^{-1}$)	N_t ($g \cdot kg^{-1}$)	C/N	C/P	$C_{mic\text{-}init.}$ ($\mu g \cdot g^{-1}$)	$N_{mic\text{-}init.}$ ($\mu g \cdot g^{-1}$)	C_{mic}/N_{mic}
Beech	2.78	17.2	59.5	3.00	3.68	251	12.9	19.6	257	3686	438	8.44
		(4.71)	(5.07)	(0.14)	(0.19)	(48.3)	(2.52)	(1.32)	(48,6)	(570)	(70.5)	(0.53)
Spruce	4.02	20.1	63.2	3.21	4.04	259	11.9	21.8	344	3757	381	9.94
		(8.53)	(5.6)	(0.58)	(0.62)	(54.5)	(2.39)	(1.71)	(72,4)	(629)	(75.1)	(0.60)
Mixed	3.66	18.5	63.5	2.96	3.79	280	13.4	21.1	329	3980	451	8.83
		(7.65)	(3.66)	(0.09)	(0.13)	(44.9)	(2.56)	(1.78)	(51,8)	(602)	(51.5)	(1.01)

Note: Standard deviation is given in parentheses (Values are not statistically significant at $p<0.05$ between the stands)

Temperature dependence of net N mineralization

The rates of net nitrogen mineralization increased exponentially (R^2 = 0.91-0.99) with increase in incubation temperature in the range of 1 –20°C during 12 weeks incubation period (Fig. 1). In comparison to beech and mixed species stands the rates of net N mineralization at spruce stand was slightly higher within the same temperature, resulting from higher accumulation of humus in the O_H horizon at spruce stand (Table 2).

The net N mineralization was statistically not different ($p < 0.05$) between beech and spruce in pure and mixed species cultures, which revealed no distinct effect of species-specific differences of substrate quality on net N mineralization. As the general climatic and biochemical properties of the adjacent forest floors were similar, the net N mineralization of each stand at mean annual air temperature (6.5°C) was calculated, which revealed no significant effect of vegetation type on net N mineralization at experimental site (Table 3). As a result of prevailing insignificant differences between the stands the net N mineralization and the Q_{10} values at mean of the experimental site were calculated and shown in Table 4 and Fig. 2. The general pattern of results exhibited a significant increase in net N mineralization along temperature increase at each stand. The observed increase in net N mineralization is consistent with results from individual sites, which have demonstrated significant positive relationships between temperature and net N mineralization (Van Cleve et al.

1990; Emmer et al. 1990; Bonan et al. 1991; Goncalves et al. 1994; MacDonald et al. 1995; Reich et al. 1997; Rustad et al. 2000). The temperature sensitivity of the net N mineralization in O_H horizon of the three stands varied between 2.24°C to 2.63°C per measured units. In consistent with our results, Dalias et al. (2002) reported the Q_{10} values of native N mineralization in O_H horizon of the conifer forest soils in the range of 1.69-2.48 at the incubation temperature of 10°C to 20°C.

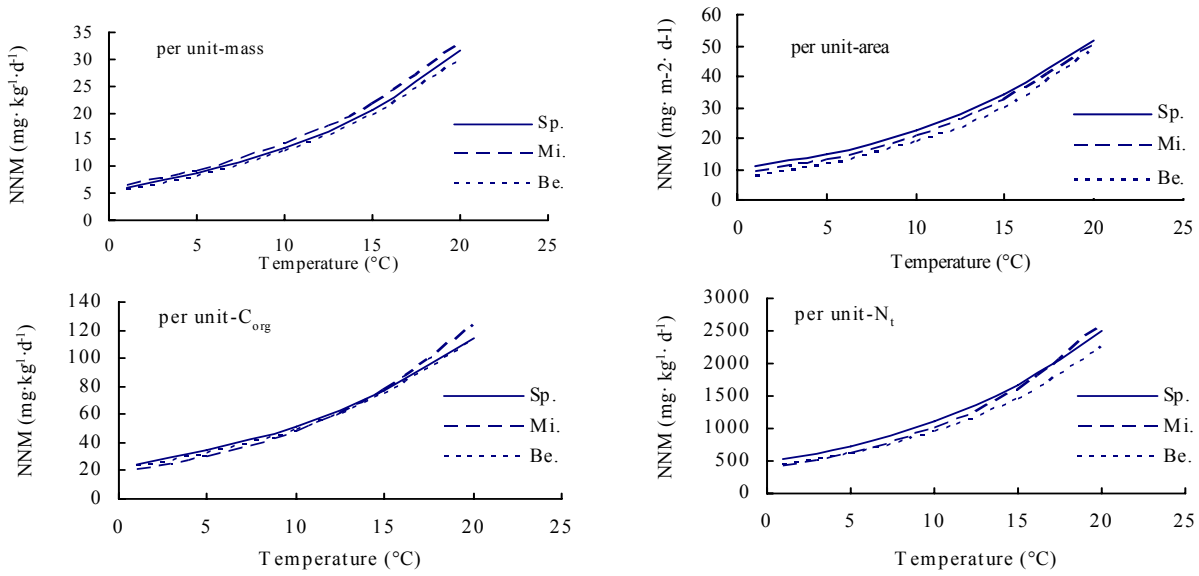

Fig. 1 Relationships between net N mineralization (per different unit) and temperature (ºC)

Table 2. Mean NNM in the O_H horizon of spruce, beech and mixed species stands along the temperature increase over the incubation period

Net N mineralization	Stand	Temperature (°C)				
		1	5	10	15	20
per unit-area	beech	5.49 (3.82)	12.1 (1.54)	17.8 (2.30)	31.8 (6.59)	47.5 (11.9)
($mg·m^{-2}·d^{-1}$)	spruce	8.08 (4.48)	16.8 (7.43)	22.1 (2.86)	35.8 (7.75)	50.9 (16.8)
	mixed	7.04 (2.61)	15.9 (5.99)	20.3 (4.46)	31.6 (4.95)	50.8 (5.67)
per unit-mass	beech	3.08 (2.09)	7.06 (1.70)	12.0 (2.91)	24.9 (4.64)	27.5 (4.88)
($mg·kg^{-1}·d^{-1}$)	spruce	3.14 (1.73)	6.48 (2.49)	16.4 (0.97)	22.9 (8.89)	30.0 (8.16)
	mixed	2.52 (0.92)	9.52 (3.21)	15.4 (6.44)	24.0 (4.70)	31.4 (8.36)
per unit-C_{org}	beech	11.7 (6.00)	30.0 (1.90)	47.7 (6.19)	91.6 (7.68)	106 (13.8)
($mg·kg^{-1}·d^{-1}$)	spruce	11.5 (4.16)	31.4 (13.1)	56.5 (7.71)	89.8 (19.7)	108 (21.8)
	mixed	8.66 (3.13)	33.8 (9.19)	52.5 (13.1)	79.5 (14.4)	122 (22.9)
per unit-N_t	beech	238 (120)	586 (62.5)	873 (123)	1820 (226)	2086 (288)
($mg·kg^{-1}·d^{-1}$)	spruce	278 (97.2)	627 (243)	1166 (128)	2030 (540)	2311 (363)
	mixed	200 (66)	670 (172)	1047 (256)	1670 (271)	2532 (467)

Notes: Standard deviation is given in parentheses

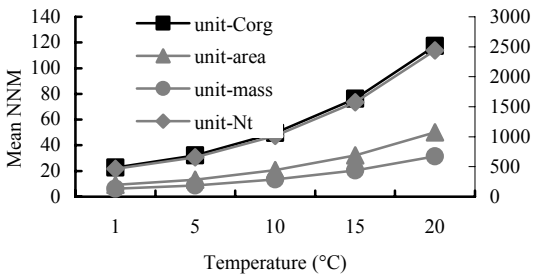

Fig. 2 Relationships between mean net N mineralization of the three stands (per different unit) and temperature (ºC), (Right Y axis is the NNM per N_t)

The Q_{10} coefficients obtained for organic matter nitrogen under different vegetation types at this experiment can be considered as indicative of the sensitivity to temperature of different quality resources. Campbell et al. (1984) suggested that Q_{10} values may be related to the degree of degradation of the soil organic matter and that more labile substances are more sensitive to temperature than those which are more refractory. Vigil and Kissel (1995) also found larger Q_{10} values for leaves with lower C/N ratios compared with leaves with less easily usable C source.

The present results confirm the hypothesis proposed by Campbell et al. (1984) since the Q_{10} values for Norway spruce

were insignificantly smaller than for beech net N mineralization data, resulting from different C/N values between spruce and beech in the magnitude of forest floor (24.2 for spruce vs. 20.9 for beech) and O_H layer (21.8 for spruce vs. 19.6 for beech). Post et al. (1985) and Anderson (1991), (1992) suggest that responses of humus net N mineralization to temperature elevation may not only be dependent on the nitrogen storage and organic matter stability in this horizon but also on the relationship between temperature response function and organic matter recalcitrance.

The warming-induced experiment at the present study on forest floor O_H at Solling forest which is already impacted by elevated atmospheric N deposition (20–40 $kg \cdot ha^{-1} \cdot a^{-1}$) increases the internal N production as reflected by the values of net N mineralization can lead to or further exacerbate conditions of "N saturation", where the input of N equals or exceeds the ability of the forest floor O_H to assimilate the added nitrogen.

Table 3. Parameters of the Q_{10} regressions fitted on the NNM data

Net N mineralization	stand	b_0	b_1	R^2	Q_{10}	NNM at 6.5°C
per unit-area	beech	7.00	0.10	0.99	2.63	13.1
($mg \cdot m^{-2} \cdot d^{-1}$)	spruce	9.95	0.08	0.99	2.28	17.0
	mixed	8.39	0.09	0.99	2.46	15.1
per unit-mass	beech	5.34	0.09	0.91	2.37	9.36
($mg \cdot kg^{-1} \cdot d^{-1}$)	spruce	5.67	0.09	0.94	2.36	9.91
	mixed	6.02	0.08	0.95	2.34	10.5
per unit-C_{org}	beech	21.0	0.08	0.93	2.32	36.3
($mg\ kg^{-1} d^{-1}$)	spruce	22.9	0.08	0.93	2.24	38.7
	mixed	18.7	0.09	0.98	2.57	34.6
per unit-N_t	beech	401	0.09	0.92	2.37	701
($mg \cdot kg^{-1} \cdot d^{-1}$)	spruce	489	0.08	0.92	2.26	831
	mixed	378	0.10	0.98	2.61	706

Note: Q_{10} values and the calculated NNM at mean of annual air temperature at Solling

Table 4. Parameters of the Q_{10} regressions fitted on the mean NNM data

Net N mineralization	b_0	b_1	R^2	Q_{10}	NNM at 6.5°C
per unit-area ($mg\ m^{-2}\ d^{-1}$)	8.43	0.09	0.99	2.44	15.1
per unit-mass ($mg\ kg^{-1}\ d^{-1}$)	5.68	0.09	0.95	2.35	9.91
per unit-C_{org} ($mg\ kg^{-1}\ d^{-1}$)	20.9	0.09	0.96	2.37	36.6
per unit-N_t ($mg\ kg^{-1}\ d^{-1}$)	423	0.09	0.96	2.40	748

Note: Q_{10} values and the calculated NNM at mean of the three stands by mean annual air temperature at Solling

Temperature dependence of microbial biomass

The values of microbial biomass C and N were not significantly different between the three stands at each temperature treatment (Table 5). Temperature increase did not change the microbial biomass significantly between 1 to 15°C at each stand dependent on the measured units. It is indicated that the increase of net N mineralization with temperature was not related to the microbial biomass carbon and nitrogen. This is in accordance with the results of the authors who found no relation between nitrogen mineralization rate and microbial biomass N to temperature variations in a beech forest soil (Bauhus et al. 1995), coniferous forest soil (Raubuch et al. 2002), and in the litter of beech and coniferous forests (Poehhacker et al. 1995; Raubuch 1998).

Table 5. Microbial C and N, C_{mic}-to-N_{mic} ratio, N_{mic}-to-N_t in O_H horizon of the three stands at different incubation temperatures, standard deviation is given in parentheses

Temp. (°C)	stand	C_{mic} ($\mu g \cdot g^{-1}$)	N_{mic} ($\mu g \cdot g^{-1}$)	C_{mic}/N_{mic}	C_{mic}/C_{org} (%)	N_{mic}/N_t (%)
1	beech	4453 (1060)	453 (146)	10.1 (1.28)	1.76 (0.23)	3.77 (0.88)
	spruce	4031 (802)	351 (76.2)	11.6 (1.83)	1.50(0.07)	3.16 (0.49)
	mixed	4234 (563)	448 (103)	9.68 (1.50)	1.58 (0.40)	4.01(1.75)
5	beech	3367 (680)	325 (63.0)	10.4 (0.38)	1.46 (0.28)	2.88 (0.50)
	spruce	3168 (845)	334 (215)	10.8 (2.94)	1.52 (0.33)	3.21 (2.05)
	mixed	3839 (538)	352 (44.9)	10.9 (0.83)	1.40 (0.13)	2.54 (0.28)
10	beech	3628 (894)	377 (111)	9.72 (0.57)	1.44 (0.17)	2.72 (0.40)
	spruce	3016 (558)	279 (63.3)	10.9 (0.98)	1.02 (0.14)	1.95 (0.33)
	mixed	3608 (760)	379(79.6)	9.56 (0.97)	1.27 (0.09)	2.66 (0.25)
15	beech	4520 (967)	484 (120)	9.42 (0.59)	1.66 (0.19)	3.54 (0.72)
	spruce	3027 (665)	286 (70.3)	10.7 (0.83)	1.21 (0.16)	2.52 (0.21)
	mixed	3616(581)	373 (71.4)	9.77 (0.63)	1.19 (0.11)	2.61(0.53)
20	beech	1950 (430)	273 (63.8)	7.19 (0.86)	0.77 (0.09)	2.14 (0.37)
	spruce	1246 (399)	150 (70.3)	9.49 (4.48)	0.46 (0.12)	1.16 (0.38)
	mixed	1900 (524)	236 (40.3)	8.02 (1.59)	0.72 (0.08)	1.92 (0.23)

Note: Values are not statistically significant at $P<0.05$ between the stands at each temperature

The C_{mic}-to-N_{mic}, C_{mic}-to-C_{org} and the N_{mic}-to-N_t (ratio of microbial nitrogen to total nitrogen） ratios remained relatively constant in the temperature range of 1–15°C with significant reducing trend at 20°C. The ergosterol content, an indicator of the fungal biomass, was not affected by temperature but the values were significantly lower under spruce in contrast to the beech and mixed species stands as related to units mass and C_{org} (Table 6). This result is in contrast with the survey of fungal-to-bacterial ratios of the forest soils in north Germany which indicates higher ratios under coniferous forests. Blagodatskaya and Anderson (1998) found fungal-to-bacterial ratios of 90-to-10 and 94-to-6 in forty spruce and beech stands with low soil $pH_{(KCl)}$ of about 3. At higher soil $pH_{(KCl)}$ of 6 much lower ratios of 84-to-16 and 74-to-26 were found for spruce and beech stands. An explanation for this discrepancy could be the lime application at the spruce stand in 1989. Liming changed the soil chemical state only for a couple of years but has obviously a long-lasting effect on the microbial community. Significant changes in the microbial status occurred above 15°C, a soil temperature which is normally not passed at Solling. The microbial biomass C and N, which were constant between 1 and 15°C, significantly decreased at 20°C at the three stands. Kleber et al. (1998) suggest that significant

changes in microbial biomass can be contributed to the changes in turnover rates of the microbial biomass at the highest temperature treatment. The microbial biomass C contributed 1.02%–1.76 % of total organic carbon in O_H horizon in the temperature range of 1–15°C, while the ratio decreased to 0.46%–0.77 % at 20°C, exhibited a restricted resource availability for decomposers, as reflected in significant decrease of microbial biomass (Fig. 3). In contrast to bacteria the ergosterol content was not significantly affected at the temperature of 20°C, although a trend to lower concentrations occurred per units mass and C_{org} at beech and mixed species stands. As a consequence, the temperature effect on the ergosterol-to-C_{mic} ratios was higher at spruce stand and reached the same values as was found for beech and mixed species stands.

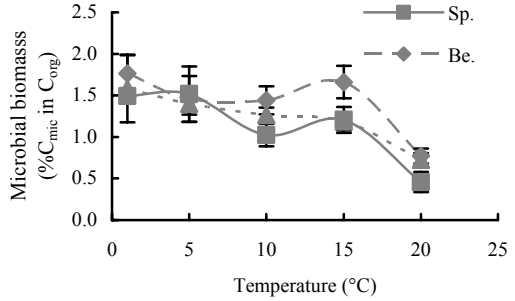

Fig. 3 Microbial biomass (% C_{mic} in C_{org}) in O_H layer of the forest floors affected by temperature

Table 6. Ergosterol content of fungal biomass in O_H horizon of the forest floor of the three stands at different incubation temperatures

Fungal ergosterol	stand	Temperature (°C)				
		1	5	10	15	20
per unit-mass	beech	27.2 (7.02)	28.8 (12.0)	32.1 (9.21)	31.1 (7.31)	22.3 (4.78)
($\mu g \cdot g^{-1}$)	spruce	15.5 (6.60)	9.83 (2.36)	14.0 (5.34)	10.0 (3.40)	12.3 (5.22)
	mixed	24.1 (3.73)	24.2 (2.90)	25.4 (6.13)	29.4 (8.86)	21.2 (3.15)
per unit-C_{org}	beech	112 (21.1)	119 (22.4)	127 (18.0)	114 (17.4)	88.5 (10.7)
($\mu g \cdot g^{-1}$)	spruce	55.8 (16.3)	47.9 (13.5)	46.8 (14.6)	38.8 (9.54)	44.1 (15.0)
	mixed	87.4 (7.6)	89.0 (11.7)	88.7 (9.36)	96.5 (24.0)	82.9 (10.7)
per unit-C_{mic}	beech	6.16 (1.15)	8.58 (3.17)	8.88 (1.50)	6.81 (0.94)	11.7 (2.41)
($\mu g \cdot mg^{-1}$)	spruce	3.77 (1.16)	3.35 (1.38)	4.42 (1.44)	3.29 (1.07)	11.2 (7.75)
	Mixed	5.81 (1.46)	6.36 (0.67)	7.06 (1.03)	8.16 (2.03)	11.8 (3.20)

Note: Standard deviation represent in parentheses.

Specific net N mineralization affected by temperature

The specific net N mineralization of the three stands was calculated assuming a linear change in microbial biomass over the incubation period. NNM/C_{mic} increased significantly from 0.61 to 11.9 ($\mu g \cdot mg^{-1} \cdot d^{-1}$) between 1 to 20°C without significant differences between the stands (Table 7). A high correlation (R^2 > 0.98) between specific NNM and temperature elevation indicated that the decline in bacterial biomass at 20°C seems to have negligible influence on the specific net N mineralization. This result was not expected for three reasons. A decline in microbial biomass at 20°C provides an easy available substrate for decomposition and a lower microbial biomass immobilize less nitrogen. A higher proportion of fungal biomass should reduce the N immobilization due to the higher C/N ratio compared to bacteria (Killham 1994). The higher energy metabolism to maintain the lower microbial biomass at 20°C may have reduced the growth and N immobilization. There might be two reasons for this discrepancy, either the microbial biomass has not been changed linear during incubation or the total effect on net N mineralization listed above were negligible.

Table 7. Specific NNM in O_H horizon of the three stands at different temperatures

Stand	NNM/C_{mic} ($\mu g \cdot mg^{-1} \cdot d^{-1}$)				
	1ºC	5 ºC	10 ºC	15 ºC	20 ºC
Beech	0.72 (0.43)	1.99 (0.40)	3.25 (0.53)	5.95 (0.52)	9.53 (1.48)
Spruce	0.80 (0.37)	1.88 (0.78)	4.87 (0.36)	6.81 (2.43)	11.9 (2.24)
Mixed	0.61 (0.19)	2.40 (0.71)	3.94 (1.28)	6.28 (1.12)	10.5 (2.31)

Standard deviation is given in parentheses

Conclusion

Comparing the exponential function curves, describing the effect of temperature on net N mineralization, revealed similar pattern in net N mineralization and correspond with a uniform microbial biomass and ergosterol content between the O_H layers of the three stands. The similar Q_{10} values between the stands suggest that the relative activity of microorganisms might be influenced more by abiotic factors such as temperature than biotic factors such as microbial species and substrate quality when no significant differences was detected in chemical properties of O_H layer. The effect of biological processes on temperature studied in this experiment is generally be characterized by Q_{10} (2.35–2.44) per measured units. Incubation studies at 1, 5, 10, 15 and 20°C revealed a similar increase in NNM in the O_H layer of beech and Norway spruce in pure and mixed species stands. Although net N mineralization followed an exponential increase to 20°C, a drastic decrease to about 50% in microbial biomass was observed compared to the lower temperatures. This was attributed to a decrease in bacterial biomass compared to a moderate decrease in fungal biomass, which was slightly higher at the beech stand than at the Norway spruce stand with respect to temperature elevation. Despite a large number of studies on biochemical processes in forest floors affected by temperature elevation, there are still not enough indications of the effect of temperature on the rates of humus development. Hence, still further studies are required to provide a better understanding of the contribution of temperature as a key control factor on nitrogen transformations in European forests.

Acknowledgement
We thank D. Gaschick for cooperating in the field and the staff of the central laboratory of the institute of soil science and forest nutrition for assistance in chemical analysis. The research study

was financially supported by Institut für Bodenkunde und Waldernährung, Georg-August-Universität, Göttingen, Germany.

References

Aber JD, Melillo JM. 1991. *Terrestrial Ecosystems*. Florida: Rinehart and Winston Inc, P.430.

Anderson TH. 2003. Microbial eco-physiological indicators to asses soil quality. *Agriculture, Ecosystems and Environment,* 2003 – *Elsevier*, **98**(1-3): 285–293.

Anderson, JM. 1991. The effects of climate change on decomposition processes in grassland and coniferous forests. *Ecological Applications*, **1**: 326–347.

Anderson JM. 1992. Responses of soils to climate change. *Advances in Ecological Research*, **22**: 163–210.

Bauhus J, Barthel R. 1995. Mechanisms for carbon and nutrient release and retention in beech forest gaps II. The role of soil microbial biomass. *Plant and Soil*, **168–169**: 585–592.

Berg B, McClaugherty C. 2003. Plant litter. Decomposition, humus formation, carbon sequestration, in: Springer Verlag, Heidelberg, Berlin.

Blagodatskaya EV, Anderson TH. 1998 Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and qCO_2 of microbial communities in forest soils. *Soil Biol and Biochem*, **30** (10-11): 1269–1274.

Bonan GB, Van Cleve K. 1991. Soil temperature, nitrogen mineralization, and carbon source-sink relationships in boreal forests. *Can. J. Forest Res*, **22**: 629–639.

Brookes PC, Landman A, Pruden G, Jenkinson DS. 1985. Chloroform fumigation and the release of soil nitrogen. *Soil Biol and Biochem*, **17**: 837–842.

Buberl HG, v Wilpert K, Trefz-Malcher G, Hildebrand EE, Wiebel M. 1994. *Der chemische Zustand von Waldböden in Baden-Württemberg*. Baden-Württemberg: Mitteilungen der Versuchs- und Forschungsanstalt, **p**182.

Campbell CA, Jame YW, Winkleman GE. 1984. Mineralization rate constants and their use for estimating nitrogen mineralization in some Canadian prairie soils. *Can J Soil Sci*, **64**: 333–343.

Dalias P, Anderson JM, Bottner P, Couteaux MM. 2002. Temperature responses of net nitrogen mineralization and nitrification in conifer forest soils incubated under standard laboratory conditions. *Soil Biol and Biochem*, **34**: 691–701.

De Neve S, Pannier J, Hofman G. 1996. Temperature effects on C- and N-mineralization from vegetable crop residues. *Plant and Soil*, **181**: 25–30.

Djajakirana G, Joergensen RG, Meyer B. 1996. Ergosterol and microbial biomass relationship in soil. *Bio Fert Soils*, **22**: 299–304.

Ellert BH, Bettany JR. 1992. Temperature dependence of net nitrogen and sulfur mineralization. *Soil Sci Soc Amer J*, **56**: 1133-1141.

Emmer IM, Tietema A. 1990. Temperature-dependent nitrogen transformation in acid oak-beach forest litter in the Netherlands. *Plant and Soil*, **122**: 193–196.

Goncalves JLM, Caryle JC. 1994. Modelling the influence of moisture and temperature on net nitrogen mineralization in a forested sandy soil. *Soil Biol and Biochem*, **26**: 1557–1564.

Hallbäcken L, Tamm CO. 1986. Changes in Soil Acidity from 1927 to 1982-1984 in a Forest Area of South-West Sweden. *Scand J Forest Res*, **1**: 219–232.

Heisner U, Wilpert Kv, Hildbrand EE. 2003. Vergleich aktueller Messungen zum Aziditätsstatus südwestdeutscher Waldböden mit historischen Messungen von 1927. *Allg Forst Jagdztg*, **174**: 2-3, 41–49.

Hobbie SE. 1996. Temperature and plant species control over litter decomposition in Alaskan tundra. *Ecological Monographs*, **66**: 503–522.

Jansson PE, Berg B. 1985. Temporal variation of litter decomposition in relation to simulated soil climate. Long-term decomposition in a scots pine forest. *Can J Bot*, **63**: 1008–10160.

Joergensen RG. 1996. The fumigation-extraction method to estimate soil microbial biomass. *Soil Biol and Biochem*, **28**: 25–31.

Killham K. 1994. *Soil Ecology*. Cambridge: Cambridge University press, p 242.

Kladivko EJ, Keeney DR. 1987. Soil nitrogen mineralization as affected by water and temperature interactions. *Biol Fert Soils*, **5**: 248–252.

Lioyd J, Taylor JA. 1994. On the temperature dependence of Soil respiration. *Func Ecol*, **8**: 315–323.

MacDonald NW, Zak DR, Pregitzer KS. 1995. Temperature effects on kinetics of microbial respiration and net nitrogen and Sulphur mineralization. *Soil Sci Soc Amer J*, **59**: 233–240.

Nadelhoffer KJ, Giblin AE, Shaver GR, Laundre JA. 1991. Effects of temperature and substrate quality on element mineralization in six Arctic soils. *Ecology*, **72**: 242–253.

Poehhacker R, Zech W. 1995. Influence of temperature on CO_2 evolution, microbial biomass C and metabolic quotient during the decomposition of two humic forest horizons. *Biol Fert Soils*, **19**: 239–245.

Post WM, Pastor J, Zinke PJ, Stangenberger AG. 1985. Global patterns of soil nitrogen storage. *Nature*, **317**: 613–616.

Raich JW, Schlesinger WH. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus*, **44**: 81–89.

Raubuch M. 1998. The impact of moisture and temperature on adenylate content and adenylate energy of microbial communities in the litter of coniferous forest soils. *Verhandlungen der Gesellschaft für Ökologie*, **28**: 415–419.

Raubuch M, Joergensen RG. 2002. C and net N mineralisation in a coniferous forest soil: the contribution of the temporal variability of microbial biomass C and N. *Soil Biol and Biochem*, **34** (6): 841–849.

Reich PB, Grigal DF, Aber JD, Gower. 1997. Nitrogen mineralization and productivity in 50 hardwood and conifer stands on diverse soils. *Ecology*, **78**: 335–347.

Rustad LE, Melillo JM, Mitchell MJ, Fernandez IJ, Steudler PA, McHale PJ. 2000. Effects of soil warming on C and N cycling in northern U.S. forest soils. In: Micker, R., Birdsey, R., Hom, J. (eds), *Responses of northern U.S. forests to environmental change*. New York: Springer Berlin, Heidelberg, New York, pp 357–381.

Sprent JI 1987. *The ecology of the nitrogen cycle*. Cambridge: University Press.

Tiktak A, Bredemeier M, Van Heerden K. 1995. The Solling dataset: Site characteristics, monitoring data and deposition scenarios. *Ecological Modelling*, **83**: 17–34.

Ulrich B. 1994. Nutrient and acid-base budget of central European forest ecosystems. In: Godbold, DL., Hüttermann, A. (eds), *Effects of Acid Rain on Forest Ecosystems*. New York: John Wiley, pp 1–50.

Van Cleve, L, Oechel, WC, Hom, JL. 1990. Response of black spruce (Picea mariana) ecosystems to soil temperature modification in interior Alaska. *Can J Forest Res*, **20**: 1530–1535.

Vance ED, Brookes PC, Jenkinson DS. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol and Biochem*, **19**: 703–707.

Vigil mF, Kissel DE. 1995. Rate of nitrogen mineralized from incorporated crop residues as influenced by temperature. *Soil Sci Soc of Amer J*, **59**: 1636–1644.

Walbridge MR, Vitousek PM. 1987. Phosphorous mineralization potentials in acid organic soils: processes affecting $^{32}PO_4$ isotope dilution measurements. *Soil Biol and Biochem*, **19**: 709–717.

Wollf B, Riek W. 1997. Deutscher Waldbodenbericht 1996.BMELF, Bd 1, und Bd 2..

Zezschwitz E. 1980. Analytische Kennwerte typischer Humusformen westfälischer Bergwälder. Zeitschrift für Pflanzenernährung and Bodenkunde, **143**: 692–700.